Clinical Investigation

Immunoresponsiveness in Ulcerative Colitis and Crohn's Disease—Effect of Colectomy and Suppression of Disease Activity

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We evaluated the effect of medically induced symptomatic disease improvement on in vitro tests of cell-mediated immune responses in 33 patients with Crohn's disease. When results obtained in 17 patients with ulcerative colitis were compared with those of 10 patients with ulcerative colitis who had undergone a colectomy, no significant correlation was detected between individual clinical and laboratory variables or the Crohn's disease activity index and in vitro tests of cell-mediated immunity. A different pattern emerged from the longitudinal tests of cell-mediated immunity: when these test results were initially abnormal in patients with Crohn's disease, clinical improvement as assessed by the Crohn's disease activity index was associated with normalizing cell-mediated immunity. In contrast, when the test results were initially normal, clinical improvement was not associated with any change in the immune response. Following colectomy in patients with ulcerative colitis, some abnormalities of suppressed immune responses remained, although patients were cured of their disease. Factors other than clinical disease activity may be responsible for the suppressed immunoresponsiveness in some patients with inflammatory bowel disease, and variable changes in cell-mediated immunity occur after both surgical and medical treatment.

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It is possible to show abnormalities in immunologic test results in varying proportions of patients with inflammatory bowel disease.[1-5] The role of these abnormalities in the etiology and pathogenesis of ulcerative colitis and Crohn's disease remains unclear, however, and it is unknown whether these changes are primary or secondary to the underlying inflammatory process, the treatment of the disease, or the presence of associated nutritional deficiencies.[6] We have previously found evidence of depressed cell-mediated immunity in most patients with inflammatory bowel disease, based on results of in vitro immunologic testing.[1] We have now done serial tests of the function of thymus-derived lymphocytes and K cells in patients with Crohn's disease and ulcerative colitis, before and after treatment resulting in improvement in clinical condition.

Materials and Methods

Patients

A total of 60 patients were studied; half were admitted to hospital and half were seen as outpatients. Standard clinical, radiologic, and histologic criteria were used to diagnose Crohn's disease in 33 patients and ulcerative colitis in 27.[7] There were equal numbers of men and women; the mean age was 30 years, with a range of 16 to 75 years. Age, sex, and frequency distribution of Crohn's disease and ulcerative colitis were similar to those reported in other studies.[8-10] All the patients were ambulatory and not currently in hospital, and none were considered clinically malnourished or to have hypoalbuminemia or lymphopenia. The patients were on standard regimens of treatment: 50% were receiving sulfasalazine (Salazopyrine) plus prednisone; 20% were receiving either sulfasalazine or prednisone alone. No patients were receiving azathioprine or other immunosuppressive drugs. At the time of initial testing of cell-mediated immunity, the patients had mild to moderate disease activity, as reflected by a mean Crohn's disease activity index of 168. The dose of prednisone or sulfasalazine was modified up or down, in accordance with the guidelines established in the National Cooperative Crohn's Disease Study so as to achieve improved ratings on the Crohn's disease activity index. While the dose of medication was modified over the three-month interval, no new medications were introduced. In some patients the administration of prednisone and sulfasalazine was discontinued between the beginning and the end of the study. When evaluating the changes in cell-mediated immunity, the therapeutic status of each patient was analyzed at the time of each individual testing. None of the patients had undergone a surgical procedure within one year, and none had clinical or laboratory evidence of liver or renal disease. Of the 27 with ulcerative colitis, 10 had undergone a colectomy two to ten years previously and currently had normally functioning ileostomies and were on no medication regimen.

Six patients with ulcerative colitis were followed for three months, with clinical disease activity decreasing during this time as a result of medical management, and 23 patients with Crohn's disease were similarly followed. These patients

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ABBREVIATIONS USED IN TEXT
Con A = concanavalin A
FCS = fetal calf serum
PHA = phytohemagglutinin
SK-SD = streptokinase-streptodornase

formed the basis of a subset of subjects who were investigated over a time span of three months to assess the effects of medical treatment and disease remission on in vitro testing of cell-mediated immunity.

The activity of the inflammatory bowel disease was assigned a score according to the method of Best and colleagues.[11] From a subset of a number of variables fulfilling a combination of constraints, these workers used a multiple-regression computer program to derive an equation for the prediction of a physician's overall ratings of how well a patient with Crohn's disease was doing. This equation, numerically simplified and using selected predictor variables, is the Crohn's disease activity index. Most of the predictor variables, such as number of liquid or soft stools, the presence of abdominal pain, general well-being, and extraintestinal complications, are experienced with at least equal frequency in chronic ulcerative colitis, whereas other predictor variables, such as the presence of an abdominal mass or perianal disease, are distinctly less common. For convenience, an activity index was calculated by the same method for both Crohn's disease and chronic ulcerative colitis, but these indices are not directly comparable. Furthermore, it is not yet possible in ulcerative colitis to designate a numeric value for the activity index representing mild, moderate, or severe disease. The patients with ulcerative colitis who had undergone colectomies had "inactive" disease because the disease process had been removed surgically.

Cellular Immune Studies

The cellular immune study techniques have been published previously.[1] Lymphocytes were isolated by centrifugation on Ficoll Hypaque[12] and washed three times in M199 with 10% fetal calf serum (FCS). Cells were cultured in pooled AB serum. Laboratory test results were reported three months later in 16 patients when the Crohn's disease activity index had fallen from a mean of 168 to 105.

Lymphocyte Transformation

Lymphocyte concentrations were adjusted to 1×10^6 cells per ml for streptokinase-streptodornase (SK-SD), these being optimum for our laboratory. Cells were suspended to RPMI 1640 (Grand Island Biological Col, Burlington, Ontario); cell suspensions were distributed into flat-bottomed microtiter plates. Mitogens or antigens were added to cell suspensions in triplicate.[13,14] The cells were cultured for 144 hours for both mitogens and antigens. Six hours before harvesting, 10 μl of tritiated thymidine was added to the cultures (concentration 20 Ci per ml, specific activity 40 mCi per nmol). Viability of cultures was routinely determined by the uptake of trypan blue stain in the remaining cells and by checking the yield of lymphocytes in unstimulated cells in parallel with stimulated cells. The cells were harvested using a multisample, semi-automated harvester (Skatron, Lierbyen, Norway).

Cultures of unstimulated cells from the same patient were made at all times to determine the rate of spontaneous transformation. The optimal concentrations of mitogens and antigens for lymphocyte transformation in our laboratory have previously been determined in a random selection of six normal subjects and six subjects with inflammatory bowel disease. In these groups there was no discernible difference in optimum doses, which were phytohemagglutinin (PHA; Difco Laboratories, Detroit), 14 μg per ml; concanavalin A (Con A; Calbiochem, Los Angeles), 45 μg per ml; streptokinase-streptodornase (Lederle Products, Montreal), 24 to 45 units per ml.

After harvesting, the fiber discs were removed, placed in 10 ml of scintillation fluid, and the emissions counted in a Beckman L.S. 120 scintillation counter. Results were expressed as the log of the ratio between stimulated and unstimulated cells from the same subject.[15]

K-Cell Activity

Targets. A monolayer of human amnion embryo cells was infected with herpes simplex virus type 1 and harvested at 24 hours. Normal, uninfected cells and infected cells were removed from the culture flask with 0.25% trypsin, then washed and labeled with chromium 51 for two hours at 37°C. Targets were washed four times in Ca^{2+} and Mg^{2+} free Hanks solution with 10% FCS[16,17]; 25×10^6 lymphocytes were incubated with 25×10^4 targets in the presence of 0.1 ml of serum from a herpes simplex plus control for three hours at 37°C. After incubation, 1 ml of cold media was added to neutralize the mix. The tubes were centrifuged and the supernatant decanted. The supernatant and pellet were counted and specific ^{51}Cr release was calculated after correction for the mean background release—about 5%.

Statistical methods. The statistical significance of the difference between the means of groups was determined by the method of paired and unpaired samples. Analysis of variance was done by multiple range and student Newman-Keuls procedures. The correlation coefficient and discriminant function were also computed.[18(p191),19]

The normal range of values of the many in vitro tests of cell-mediated immunity was determined in a group of 66 healthy control personnel. The results of tests of cell-mediated immunity in patients with idiopathic ulcerative colitis or Crohn's disease were compared with these normal values. For any given test, the magnitude of abnormalities was assessed by comparing the mean values of normal subjects with the mean value of the group with idiopathic ulcerative colitis or Crohn's disease. Because of the nongaussian distribution of the results of the test of lymphocyte transformation, these results were expressed logarithmically.[15]

On a day-to-day basis, repeated testing of patients over a one- to three-day span or examining the same patient's cells in triplicate or quadruplicate showed that the variation was small within either the normal or the abnormal range.

Results

Mitogen and Antigen T-Cell Proliferation

Lymphocyte transformation responses to PHA were significantly reduced in patients with unoperated ulcerative colitis and Crohn's disease when compared with normal subjects (Table 1). In the case of ulcerative colitis patients with colectomies, this abnormality was reversed. With the mitogen Con A, the suppression in transformation responses was notable in all three groups when compared with normal subjects. In contrast to the PHA responses, there was no difference between the ulcerative colitis and the ulcerative

TABLE 1.—*In Vitro Testing of Immunoresponsiveness in Control Subjects, Patients With Ulcerative Colitis, and Patients With Ulcerative Colitis Following Panproctocolectomy (Mean ± Standard Error of the Mean)*

Test, log	Control Subjects, N=66	Ulcerative Colitis, Colectomy, N=10	Ulcerative Colitis, No Colectomy, N=17	Crohn's Disease, N=33
Phytohemagglutinin				
50	1.32±0.07	1.53±0.1	1.11±0.17†	0.86±0.18‡
100	1.43±0.07	1.64±0.1	1.17±0.10†	1.20±0.12‡
Concanavalin A				
50	1.06±0.12	0.70±0.22	0.63±0.18	0.64±0.13
100	0.97±0.15	0.37±0.23*	0.70±0.19	0.48±0.15
Streptokinase-streptodornase				
50	0.54±0.08	0.04±0.13*	0.24±0.09	0.11±0.12
100	0.47±0.09	0.07±0.10*	0.15±0.10	0.02±0.10
K-Cell activity	44.8±2.5	45.7±7.9	46±4.9	42±3.48

*The mean of the value of control subjects was statistically different from the mean value in the ulcerative colitis-colectomy group ($P<.05$).
†The mean value in the ulcerative colitis-colectomy group was statistically different from the mean value in the ulcerative colitis-no colectomy group ($P<.05$).
‡The mean value in the ulcerative colitis-colectomy group was statistically different from the mean value in the Crohn's disease group ($P<.05$).

TABLE 2.—*Repeat In Vitro Testing of Immunoresponsiveness in Patients With Ulcerative Colitis and Crohn's Disease (Mean ± Standard Error of the Mean)*

	Ulcerative Colitis			Crohn's Disease		
Test, log	Patients, No.	First Testing	Second Testing	Patients, No.	First Testing	Second Testing
Phytohemagglutinin						
50	6	1.0±0.33	0.9±0.33	23	0.33±0.3	1.03±0.25*
100	6	1.1±41	1.1±19	23	0.73±0.22	1.43±0.18*
Concanavalin A						
50	6	0.9±0.33	1.1±0.33	23	0.51±0.28	1.45±0.26*
100	6	0.8±0.49	1.1±0.29	23	0.24±0.37	1.58±0.26*
Streptokinase-streptodornase						
50	6	0.5±0.22	0.6±0.45	19	0.11±0.20	0.31±0.29
100	5	0.3±0.35	0.9±0.65	10	0.18±0.23	0.40±0.20
K-Cell activity	5	30.4±12.0	33.6±8.13	10	47.7±5.38	32.5±4.10*

*The mean value in the second testing was statistically different from the mean value in the first testing ($P<.05$).

colitis with colectomy groups. Similar trends were noted with the antigen SK-SD. Substantial suppression of transformation responses was confirmed in active ulcerative colitis and Crohn's disease, with the degree of abnormality most noticeable in those with Crohn's disease. In addition, these results show that in two of the three stimuli (Con A and SK-SD), the suppression in the response seen in active ulcerative colitis disease was not normalized by colectomy.

K-Cell Activity

No suppression of K-cell activity was noted in any of the three groups tested when compared with normal subjects (Table 1). No differences were noted between those with ulcerative colitis with or without colectomies.

Serial Studies

The difference between the first (baseline) and second (longitudinal) testing in 6 patients with ulcerative colitis and 23 patients with Crohn's disease after at least three months of disease-suppressive therapy is shown in Table 2. The suppressed responses were initially more profound in the selected patients with Crohn's disease when compared to the selected ulcerative colitis patients. In those subjects with ulcerative colitis, a tendency to normalization of suppressed responses was noted, and although in the small number studied there was no statistically significant difference between the first and second testing, levels in the normal range were seen at second testing with both Con A and SK-SD.

In distinct contrast, the more profound abnormalities noticed at first testing in patients with Crohn's disease were reversed to a statistically significant level at second testing ($P<.05$), reaching normal levels with both Con A and SK-SD. In these patients, K-cell activity was also reduced at second testing.

The results of T-cell stimulation in patients with Crohn's disease were further analyzed by subdividing the data into two groups: those subjects whose tests were initially abnormal and those whose tests were initially normal. Of the eight Crohn's disease patients whose PHA responses were initially low, subsequent normalization occurred in seven. Of the 15 whose PHA responses were initially normal, 14 remained normal on subsequent testing and one became abnormal (Figure 1). The increase in the initially abnormal tests was statistically significant ($P<.05$), whereas the change in the initially normal tests failed to achieve statistical significance. The same can be said for optimum concentrations of Con A. Ten patients initially had suppressed responses, with a good correlation between those patients who had a suppressed

response to Con A and PHA. Of those ten, nine had normal results at retesting and one result remained abnormal. A further 11 patients had normal responses on initial testing, and these remained normal at repeat testing.

Correlation Between Clinical and Laboratory Variables

A correlation was sought between the results of each test shown in Table 2 and a wide range of clinical and laboratory variables. In both disease groups, there was no statistically significant correlation between the disease activity index and the individual values in any of the groups of tests of cell-mediated immunity. This lack of correlation existed for both first and second testing and when only those patients with abnormal values were considered. Furthermore, analysis of the therapeutic status at the time of each individual testing—baseline and longitudinal—failed to show a clear association between the changes in the tests of cell-mediated immunity and the medical regimen used. For example, in patients initially taking prednisone, the cell-mediated immunity was no different than in those patients not taking prednisone. Furthermore, a change in the dose of prednisone over a three-month interval was not associated with a directionally consistent change in cell-mediated immunity, regardless of whether the baseline test was normal or abnormal. Thus, the altered immune cellular assays were not a function of the dose of prednisone.

Discussion

In patients with Crohn's disease and ulcerative colitis, in vitro lymphocyte transformation responses to mitogens and antigens and proportions of subpopulations of circulating lymphocytes may differ from those seen in normal persons.[1,20-22] The factors that lead to this suppression of immunoresponsiveness are not clearly defined, but there are probably several, including possibly malnutrition[6] or changes in disease activity. This study evaluates the role of colectomy in patients with ulcerative colitis and the effect of medical treatment in both ulcerative colitis and Crohn's disease by measuring in vitro lymphocyte transformation responses to mitogens and antigens and K-cell activity before and after treatment. These studies were done on peripheral blood lymphocytes rather than on gut-associated lymphoid tissue. When the functional properties of T and B cells from human mesenteric lymph nodes are compared with the properties of blood lymphocytes, there were no differences between T-cell subset fractions, but the pattern of immunoglobulin secretion of polyclonally actuated lymphocytes from mesenteric lymph nodes differed from that of peripheral blood lymphocytes in both the amount of immunoglobulin secreted and the isotope distribution.[23] We did not measure B-cell function, but it is likely that the measured changes in peripheral blood T lymphocytes reflected events that were occurring in the intestine.

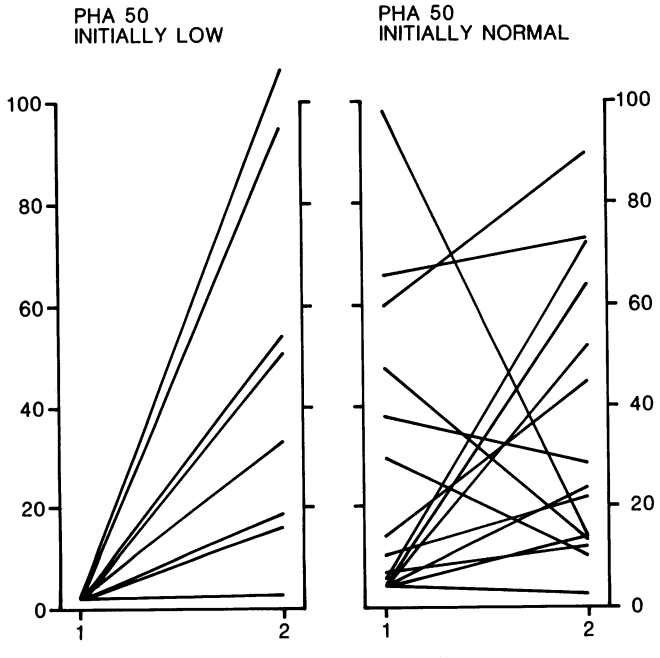

Figure 1.—Phytohemagglutinin (PHA) responses in patients with Crohn's disease. The PHA_{50} responses were determined when the patients were unwell and then on a second occasion when their symptoms had improved.

Certain trends were present that were different for individual mitogens and antigens. For example, changes that occurred with Con A and SK-SD tended to be similar in both groups of patients, whereas PHA responses tended to be different. Thus, the prevalence of suppressed immunoresponsive changes in vitro and the effects of treatment differ depending on the individual test undertaken. In addition, the level of suppression of immunoresponsiveness using such techniques was more profound in patients with Crohn's disease than in those with ulcerative colitis. The degree of change seen in our patients would suggest that the defect in immunosuppression in Crohn's disease may be a more important etiologic factor than that seen in ulcerative colitis. Such a consideration must be made cautiously, however, since in this study the baseline values in ulcerative colitis were so much closer to normal than those in patients with Crohn's disease.

In ulcerative colitis, colectomies did not appear to alter the suppressed immune responses to Con A and SK-SD, although PHA responses rose above those seen in normal persons. The suppressed responses to all three mitogens and antigens and their failure to change following colectomy suggest that although the diseased section was removed, colectomy had not eradicated the factor(s) responsible for the altered cell-mediated immunity. Alternatively, immune function may have been abnormal before colectomy and may have remained so despite removal of the diseased section.

Other workers[4] have shown that cutaneous anergy to dinitrochlorobenzene was still present after bowel resection in 6 of 10 patients (60%), while in ulcerative colitis anergy was found after colectomy in only 2 of 14 patients (14%). Tests of peripheral T and B cells studied before and after bowel resection, however, showed no difference between patients with ulcerative colitis and those with Crohn's disease.[5] The substantial abnormalities in PHA responses following colectomy observed in this study are intriguing and unexplained. Removal of the diseased bowel, weight gain, and cessation of steroid medication improve skin test reactivity but do not correct the depressed peripheral T-lymphocyte counts.[5] It was not possible in this study to evaluate the role of colectomy on PHA responses, although this is a possible factor that needs to be evaluated. It was also not possible to explore questions about fundamental differences between ulcerative colitis and Crohn's disease on the basis of preoperative and postoperative data because no patients with Crohn's disease treated surgically were included in this study.

Further evidence that the therapy for active ulcerative colitis does not primarily influence the abnormal cell-mediated immunity is seen in the patients who were tested both before and after a course of medical treatment of active ulcerative colitis. In these patients, there was no significant difference between the pretreatment and posttreatment results. Others have also suggested that the immune defect in patients with ulcerative colitis may be a secondary phenomenon.[4,5]

In patients with Crohn's disease, no correlation was observed between the baseline cell-mediated immunity and the initial value of the Crohn's disease activity index. Also, at second testing, there was no correlation. There did appear to be two subgroups of patients with Crohn's disease: those with initially normal cell-mediated immunity, and those whose baseline immunity was abnormal. In examining those two subgroups separately using longitudinal tests of cell-mediated immunity, a further difference emerged: when baseline test results were abnormal, clinical improvement was associated with more normal results on the tests of cell-mediated immunity. In the small number of patients studied, the responses, while not pronounced, were suppressed, and at second testing with both Con A and SK-SD, the responses were similar to those seen in normal subjects. At second testing in patients with Crohn's disease, there was a notable rise in responses to PHA and Con A following at least three months of medical treatment (Table 2). This effect was most pronounced when patients were initially found to have a suppressed response, which usually became normal after clinical improvement (Figure 1). These results suggest that treatment resulting in suppression of the disease process is associated with a return to normal of previously suppressed immunoresponsiveness.

The improvement in the initially abnormal immune responses may have been due to the disease process because clinical improvement was associated with immune improvement (Figure 1). The patients with initially abnormal immune responses, however, were just as sick clinically as the patients with initially normal immune responses, and their doses of prednisone and their nutritional status were comparable. Furthermore, the altered immune cellular assays were not a function of the dose of prednisone. Thus, there appear to be at least two subgroups of patients with Crohn's disease. Those with initially abnormal immunity were noted to have a dramatic improvement in cell-mediated immunity associated with clinical improvement. In contrast, in those patients with Crohn's disease who initially had normal cell-mediated immunity, the immunity remained normal when the patients' clinical condition improved. We must now examine whether the clinical course or response to treatment varies in these two immunologic subgroups of patients with Crohn's disease.

No correlation was found between changes or levels of baseline immune responses and the Crohn's disease activity index; similar results have previously been reported.[1] This suggests that changes in cell-mediated immunity either toward or away from normalcy are not reflected by variations in selected clinical features of the disease. We must stress that our group of patients had only mild to moderate disease activity, and that more substantial changes in the activity index might have been associated with greater changes in cell-mediated immunity. Thus, the change in the activity index in this study from a mean of 168 to 105 may show an insufficient alteration in disease activity to be reflected by a change in cell-mediated immunity. The alternative explanation is that the Crohn's disease activity index reflects factors other than those responsible for the underlying disease process and under these circumstances need not be expected to reflect primary etiologic factors in the pathogenesis of the disease.

Indeed, the topic of the assessment of Crohn's disease activity has recently been reviewed,[11] and the use of the Crohn's disease activity index has been criticized.[4] In claiming a favorable response to the use of prednisone and sulfasalazine as assessed by their effect on the Crohn's disease activity index, a nonspecific effect on at least four of the eight variables used in the index cannot be totally dismissed as a possible explanation.[13] The measurement of serum albumin and seromucoid has been suggested to be of use in distinguishing between the extremes of the clinical states of relative health and well-being.[5] The serum albumin concentrations were all greater than 3.5 grams per dl in our patients with Crohn's disease, and measurements of serum seromucoid were not available to us.

We conclude that factors other than just the disease activity are responsible for the suppressed immunoresponsiveness in patients with inflammatory bowel disease and that variable changes in this in vitro test occur subsequent to both surgical and medical treatment.

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